

a.) Amendments to the Claims

1. (Cancelled)

2. (Previously Presented) A method for quantitatively determining LDL cholesterol in a biological sample, which comprises:

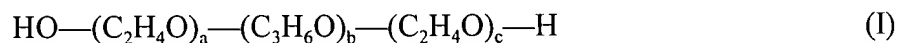
(I) reacting cholesterol in the presence of:

- a) a biological sample,
- b) CH enzymes selected from the group consisting of (i) a combination of cholesterol esterase and cholesterol oxidase and (ii) a combination of cholesterol esterase, cholesterol dehydrogenase and oxidized coenzyme, and
- c) a polyoxyethylene derivative and a polyoxyethylene-polyoxypropylene copolymer which enable the CH enzymes to act only on LDL cholesterol to form hydrogen peroxide or reduced coenzyme; and

(II) measuring the amount of the hydrogen peroxide or reduced coenzyme.

3. (Currently Amended) The method according to claim [(2)] 2, wherein the polyoxyethylene derivative is a polyoxyethylene alkyl ether or a polyoxyethylene alkylaryl ether.

4. (Previously Presented) The method according to claim 2, wherein the polyoxyethylene-polyoxypropylene copolymer is a surfactant represented by formula (I):



wherein a, b and c independently represent an integer of 1 to 200.

5. (Currently Amended) A method for continuous fractional determination of HDL cholesterol and LDL cholesterol in a biological sample, which comprises:

- (I) subjecting cholesterol to reaction in the presence of:
 - a) a biological sample,
 - b) CH enzymes selected from the group consisting of (i) a combination of cholesterol esterase and cholesterol oxidase and (ii) a combination of cholesterol esterase, cholesterol dehydrogenase and oxidized coenzyme, and
 - c) a reagent enabling the CH enzyme to act only on HDL cholesterol to form hydrogen peroxide or reduced coenzyme,
- (II) measuring [the] an amount of [the] hydrogen peroxide or reduced coenzyme to quantitatively determine the concentration of HDL cholesterol, then adding a polyoxyethylene derivative and a polyoxyethylene-polyoxypropylene copolymer which enable the CH enzymes to act only on LDL cholesterol;
- (III) subjecting cholesterol to the reaction to form hydrogen peroxide or reduced coenzyme;
- (IV) measuring the amount of the hydrogen peroxide or reduced coenzyme to quantitatively determine the concentration of LDL cholesterol.

6. (Currently Amended) A method for continuous fractional determination of HDL cholesterol and LDL cholesterol in a biological sample, which comprises:

- (I) conducting a first reaction of cholesterol in the presence of:
 - a) a biological sample,

b) CH enzymes selected from the group consisting of (i) a combination of cholesterol esterase and cholesterol oxidase and (ii) a combination of cholesterol esterase, cholesterol dehydrogenase and oxidized coenzyme, and

c) a reagent enabling the CH enzymes to act only on HDL cholesterol to form hydrogen peroxide or reduced coenzyme, and

(II) measuring [the] an amount of [the] hydrogen peroxide or reduced coenzyme to quantitatively determine the concentration of HDL cholesterol, then adding CH enzymes, and a polyoxyethylene derivative and a polyoxyethylene-polyoxypropylene copolymer which enable the CH enzymes to act only on LDL cholesterol,

(III) conducting a second reaction of cholesterol to form hydrogen peroxide or reduced coenzyme, and measuring the amount of the hydrogen peroxide or reduced coenzyme to quantitatively determine the concentration of LDL cholesterol.

7. (Cancelled)

8. (Previously Presented) The method according to claim 5 or 6, wherein the polyoxyethylene derivative is a polyoxyethylene alkyl ether or a polyoxyethylene alkylaryl ether.

9. (Previously Presented) The method according to claim 5 or 6, wherein the polyoxyethylene-polyoxypropylene copolymer is a surfactant represented by formula (I):



wherein a, b and c, independently represent an integer of 1 to 200.

10. (Cancelled)

11. (Cancelled)

12. (Previously Presented) The method according to claim 5 or 6, wherein the reagent enabling CH enzyme to act only on HDL cholesterol is a reagent for aggregating lipoproteins other than HDL.

13. (Cancelled)

14. (Previously Presented) The method according to claim 12, wherein the reagent for aggregating lipoproteins other than HDL is a reagent comprising a divalent metal salt and at least one member selected from the group consisting of heparin or a salt thereof, phosphotungstic acid or a salt thereof, dextran sulfuric acid or a salt thereof, polyethylene glycol, sulfated cyclodextrin or a salt thereof, and sulfated oligosaccharide or a salt thereof.

15. (Previously Presented) The method according to claim 6, wherein the CH enzymes used in the first reaction are chemically modified enzymes and the CH enzymes used in the second reaction are enzymes that are not chemically modified.

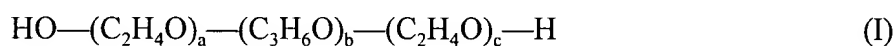
16. (Cancelled)

17. (Cancelled)

18. (Currently Amended) A reagent for determining LDL cholesterol comprising CH enzymes selected from the group consisting of (i) a combination of cholesterol esterase and cholesterol oxidase and (ii) a combination of cholesterol ~~esterase~~ esterase, cholesterol dehydrogenase and oxidized coenzyme, and a polyoxyethylene derivative and a polyoxyethylene-polyoxypropylene copolymer which enable the CH enzymes to act only on LDL cholesterol.

19. (Currently Amended) The reagent according to claim [(18)] 18, wherein the polyoxyethylene derivative is a polyoxyethylene alkylaryl ether.

20. (Previously Presented) The reagent according to claim 18, wherein the polyoxyethylene-polyoxypropylene copolymer is a surfactant represented by formula (I):



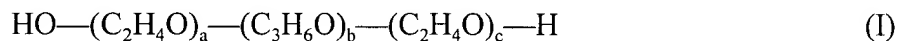
wherein a, b and c independently represent an integer of 1 to 200.

21. (Cancelled)

22. (Previously Presented) A reagent kit for continuous fractional determination of HDL cholesterol and LDL cholesterol comprising a first reagent comprising CH enzymes selected from the group consisting of (i) a combination of cholesterol esterase and cholesterol oxidase and (ii) a combination of cholesterol esterase, cholesterol dehydrogenase and oxidized coenzyme, and a reagent for aggregating lipoproteins other than HDL, and a second reagent comprising a polyoxyethylene derivative and a polyoxyethylene-polyoxypropylene copolymer which enable CH enzymes to act only on LDL cholesterol.

23. (Previously Presented) The reagent kit according to claim 22, wherein the polyoxyethylene derivative is a polyoxyethylene alkylaryl ether.

24. (Previously Presented) The reagent kit according to claim 22, wherein the polyoxyethylene-polyoxypropylene copolymer is a surfactant represented by formula (I):



wherein a, b and c independently represent an integer of 1 to 200.

25. (Cancelled)

26. (Cancelled)

27. (Previously Presented) The reagent kit according to claim 22, wherein the reagent for aggregating lipoprotein other than HDL is a reagent comprising a divalent metal salt and at least one member selected from the group consisting of heparin or a salt thereof, phosphotungstic acid or a salt thereof, dextran sulfuric acid or a salt thereof, polyethylene glycol, sulfated cyclodextrin or a salt thereof, and sulfated oligosaccharide or a salt thereof.

28. (Previously Presented) The method according to claim 2, 5 or 6, wherein the CH enzymes are cholesterol esterase and cholesterol oxidase, and the determination of hydrogen peroxide is carried out by reacting the hydrogen peroxide with chromogen in the presence of peroxidase to form a dye and measuring the absorbance of the reaction mixture.

29. (Previously Presented) The reagent according to claim 18, wherein the CH enzymes are cholesterol esterase and cholesterol oxidase, and the reagent further comprises peroxidase and chromogen which produces a dye by reaction with hydrogen peroxide in the presence of peroxidase.

30. (Previously Presented) The reagent kit according to claim 22, wherein the second reagent further comprises CH enzymes.

31. (Previously Presented) The reagent kit according to claim 22, wherein the CH enzymes are cholesterol esterase and cholesterol oxidase, and the first reagent further comprises peroxidase and chromogen which produces a dye by reaction with hydrogen peroxide in the presence of peroxidase.

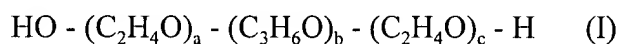
32. (Previously Presented) The reagent kit according to claim 30, wherein the CH enzymes in the first reagent are chemically modified enzymes and the CH enzymes in the second reagent are enzymes that are not chemically modified.

33. (Previously Presented) The method according to claim 12, wherein the reagent for aggregating lipoproteins other than HDL further contains a nonionic surfactant that does not solubilize the aggregated lipoproteins.

34. (Previously Presented) The reagent kit according to claim 31, wherein the CH enzymes in the first reagent are chemically modified enzymes and the CH enzymes in the second reagent are enzymes that are not chemically modified.

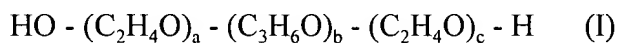
35. (Previously Presented) The reagent kit according to claim 22, wherein the reagent for aggregating lipoproteins other than HDL further contains a nonionic surfactant that does not solubilize the aggregated lipoproteins.

36. (Previously Presented) The method according to claim 2, wherein the polyoxyethylene derivative is a polyoxyethylene alkyl ether or a polyoxyethylene alkylaryl ether, and the polyoxyethylene-polyoxypropylene copolymer is a surfactant represented by formula (I):



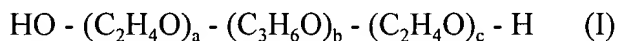
wherein a, b and c independently represent an integer of 1 to 200.

37. (Previously Presented) The method according to claim 5 or 6, wherein the polyoxyethylene derivative is a polyoxyethylene alkyl ether or a polyoxyethylene alkylaryl ether, and the polyoxyethylene-polyoxypropylene copolymer is a surfactant represented by formula (I):



wherein a, b and c independently represent an integer of 1 to 200.

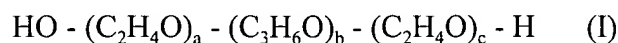
38. (Previously Presented) The method according to claim 5 or 6, wherein the polyoxyethylene derivative is a polyoxyethylene alkyl ether or a polyoxyethylene alkylaryl ether; the polyoxyethylene-polyoxypropylene copolymer is a surfactant represented by formula (I):



wherein a, b and c independently represent an integer of 1 to 200; and the reagent enabling CH enzymes to act only on HDL comprises a (i) divalent metal salt and (ii) at least one

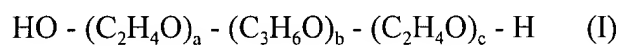
member selected from the group consisting of heparin or a salt thereof, phosphotungstic acid or a salt thereof, dextran sulfuric acid or a salt thereof, polyethylene glycol, sulfated cyclodextrin or a salt thereof, and sulfated oligosaccharide or a salt thereof.

39. (Previously Presented) The reagent according to claim 18, wherein the polyoxyethylene derivative is a polyoxyethylene alkylaryl ether, and the polyoxyethylene-polyoxypropylene copolymer is a surfactant represented by formula (I):



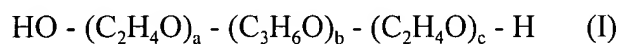
wherein a, b and c independently represent an integer of 1 to 200.

40. (Previously Presented) The reagent kit according to claim 22, wherein the polyoxyethylene derivative is a polyoxyethylene alkylaryl ether, and the polyoxyethylene-polyoxypropylene copolymer is a surfactant represented by formula (I):



wherein a, b and c independently represent an integer of 1 to 200.

41. (Previously Presented) The reagent kit according to claim 22, wherein the polyoxyethylene derivative is a polyoxyethylene alkylaryl ether; the polyoxyethylene-polyoxypropylene copolymer is a surfactant represented by formula (I):



wherein a, b and c independently represent an integer of 1 to 200; and the reagent for aggregating lipoproteins other than HDL comprises (i) a divalent metal salt and (ii) at least one member selected from the group consisting of heparin or a salt thereof, phosphotungstic acid or a salt thereof, dextran sulfuric acid or a salt thereof, polyethylene glycol, sulfated cyclodextrin or a salt thereof, and sulfated oligosaccharide or a salt thereof.